EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

Standard Operating Procedure
for
VITEK 2 Compact: Use, Maintenance and Quality Control Procedures

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1.0 SCOPE AND APPLICATION:

1.1 This protocol describes the procedures for preparation and identification of test microorganisms (test microbes and Quality Control Organisms) using the VITEK 2 Compact Instrument. The Quality Control process encompasses the annual service and certification of the instrument by bioMérieux and the annual Quality Control of one lot of Gram negative (GN), Gram positive (GP), and Bacillus (BCL) cards using the organisms listed in Table 1.

2.0 DEFINITIONS:

2.1 ATCC = American Type Culture Collection

2.2 BCL = Bacillus Card

2.3 GN = Gram Negative Card

2.4 GP = Gram Positive Card

2.5 NA = Nutrient Agar

2.6 TSA = Trypticase Soy Agar

2.7 TSB = Trypticase Soy Broth

2.8 Test Microbes = The test microbe used in either a product efficacy test (*Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis*) or research and development study.

2.9 Quality Control (QC) Organism = The organisms used to QC one lot of each type of VITEK 2 Compact cards (GN, GP and BCL) once a year. The list of QC organisms was provided by bioMérieux.

2.10 V2C = Vitek 2 Compact. The V2C is an automated identification system for microorganisms. It is used for organism identification and confirmation.

3.0 HEALTH AND SAFETY:

3.1 Laboratory personnel should follow biosafety procedures appropriate for the organisms being confirmed as outlined in SOP MB-01, Biosafety in the Laboratory.

3.2 Biohazardous spills can occur inside the V2C instrument. All organism

suspensions, cards, cassettes, test tubes, sample transfer tubes, waste bin and the user interface panel should be considered as potentially infectious. Use latex gloves when handling the cassette with live organisms.

3.3 If a spill occurs in the V2C instrument use a registered hospital disinfectant such as Lysol IC 1:200 for cleaning purposes. Use according to label instructions.

3.4 The four internal carousels should be removed and each section cleaned on a regular basis. See the Instrument User Manual for directions on cleaning the carousel.

4.0 CAUTIONS:

4.1 Suspensions not within the appropriate range on the Vitek 2 DensiChek may compromise the card performance.

4.2 A minimum of 3 mL of sterile saline must be used when filling the cards. To ensure this, the dispensette has been preset to dispense 4 mL of sterile saline into the suspension tube.

4.3 When adding information in the application window, make sure you save your data prior to logging out or continuing to prepare additional isolates. Any unsaved data will not be recovered when the inactivity time limit (set at 60 minutes) has been exceeded.

4.4 Do not use glass tubes with the Densichek as it may result in an erroneous McFarland Reading.

5.0 INTERFERENCES:

5.1 Improper subculturing and filling of VITEK cards may result in inconsistent or erroneous biopatterns.

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel are required to be knowledgeable of the procedures in this SOP. The On-Line User Manual and Instrument User Manual are maintained near the instrument. All users of the V2C must have hands on training in the use of the instrument and will be required to successfully complete one competency test using one VITEK card/organism. Documentation of training and familiarization with this SOP can be found in the training file for each

employee.

7.0 SPECIAL APPARATUS AND MATERIALS:

7.1 For QC organisms use REMEL's ready-to-use disposable Culti-Loops or ATTC lyophilized ampules. See Table 1.

7.2 Sterile Inoculating Loops

7.3 Supplemental Media: NA, TSB, TSA with 5% Sheep Blood, and TSA.

7.5 VITEK 2 Compact Identification cards (GP, BCL, and GN) store at 2-8°C in unopened original liner.

7.6 75 mm x 12 mm clear polystyrene tubes (single use only)

7.7 DensiChek Meter with 3.0 McFarland Standard for calibration

7.8 Barcoded 10 well cassette card holders

7.9 Internal Carousel for card processing

8.0 INSTRUMENT OR METHOD CALIBRATION:

8.1 Factory Calibrations: Prior to shipment, the VITEK 2 Compact Instrument met all acceptance test procedures stipulated by bioMérieux. The Field Service Engineer performed a verification of the VITEK factory calibration as a part of the installation procedure of this instrument. This can be found in the VITEK Certification Records Book.

8.2 Internal monitoring of the VITEK reader/incubator module.

8.2.1 The VITEK reader/incubator module houses the card handling and scanning mechanism as well as the heater that maintains the cards at the required incubation temperature. The trays that hold the cards are mounted to a carousel that rotates once every 15 minutes to position the cards for data scanning and identification. A thermistor is located in the center of the carousel shaft and positioned to monitor any change of temperature in the carousel stack. A heater and fan on top of the carousel maintains the temperature at an average temperature of 35.5°C.

8.2.2 The incubation temperature is automatically verified during the initiation of the VITEK instrument and computer. Temperature deviations of ±2°C generate error messages at the data terminal module such as, "Reader Temperature High," or "Reader Temperature Low." The process cycle is aborted if the temperature varies ±5°C from the set temperature for more than one hour.

8.2.3 Results for the calibration of the DensiChek instrument are digital and should be within ±0.10 of the standard being used to calibrate the instrument. If the calibration falls outside of this range, repeat the calibration steps. If the McFarland value is still outside the acceptable range, discontinue use of the DensiChek and contact bioMérieux.

8.2.4 Error Message Queue:

8.2.4.1 When the V2C instrument flashes an Error Message Queue the instrument will not operate. Each error message must be reviewed by opening each message using the down arrows on the instrument key board followed by the exclamation point. If the Error Message Queue does not clear after this procedure, shut the machine down for 2 minutes and reboot the instrument. If this fails, call technical services at bioMérieux. (personal communication with bioMérieux technical services, RS 12/05)

9.0 SAMPLE HANDLING AND STORAGE:

9.1 Initiation of QC Organisms (GN, GP and BCL):

9.1.1 Re-hydrate according to the manufacturer's instructions (Remel or ATCC).

9.1.2 Perform steak isolation of re-hydrated culture of each organism onto appropriate agar plates (see Table 1).

9.1.3 Incubate the plate at the appropriate temperature listed in Table 1.

9.1.4 Prepare isolates for long term storage as described in 9.2.

9.2 Long Term Storage of QC organisms (GN, GP and BCL):

9.2.1 Make a heavy suspension from the plates described in 9.1 into Trypticase Soy Broth with 15 % glycerol.

9.2.2 Aliquot suspension in sterile 0.5 mL cryovials.

9.2.3 Freeze the cryovials at –70° C.

9.3 Storage of test microbes or research organisms:

9.3.1 Store test or research organisms at 2-8ºC in their original tubes until a positive identification is received from the V2C.

10.0 PROCEDURE AND ANALYSIS:

10.1 Initiation of the V2C System for Test Microbes and QC Organisms:

10.1.1 The VITEK 2 Compact Instrument is always "on"; the instrument will say "Ready" or "Not Ready" on the digital screen. The V2C will not run if it is not ready. Once the computer is initialized, the instrument will say "Ready."

10.1.2 From the upper left side of the screen, select VITEK 2 Compact to initiate the system. After the system is initiated, log onto the system using the appropriate user name and password. The system is now initialized and ready for data entry.

10.2 Preparation of Test Microbes and QC Organisms:

10.2.1 For QC organisms: remove the 0.5 mL cryovials from the –70°C freezer. Avoid repeated thawing and freezing of the frozen culture by aseptically removing a small portion (or loopful) of the frozen inoculum, then immediately return cryovials to –70 °C freezer. See Section 9.1 for long term storage procedures for QC organisms.

10.2.1.1 Streak isolate the inoculum on a warmed (35-37°C) agar plate appropriate for the QC organism. See Table 1.

10.2.1.2 For QC organisms, a second streak isolation on the appropriate media is recommended. For product tests, a second streak isolation step is not required unless there is evidence of a mixed culture.

10.2.2 For "unknowns" or Test Microbes: use the tubes from carriers that failed a product or research test. See Section 9.2 for storage of test

microbes or research organisms.

10.2.2.1 Streak isolate the inoculum from a product test on TSA warmed to room temperature. See section 10.3 for incubation time.

10.3 Preparing Streak Isolation of Test Microbes or QC Organisms. (Record information on the V2C Test Microbe Transfer and Confirmation Sheet for Quality Control Organisms or the appropriate Test Microbe Confirmation Sheet – see MB-05, MB-06 or MB-07):

10.3.1 According to the Software User Manual for BCL, the organism to be identified must be a pure culture 18 to 24 hours old. Use TSA or NA to prepare isolates.

10.3.2 According to the Software User Manual for GN, the organism to be identified must be a pure culture 18 to 24 hours old. Use TSA or TSA with 5% sheep blood to prepare isolates.

10.3.3 According to the Software User Manual for GP, the organism to be identified must be a pure culture 12 to 48 hours old. Use TSA or TSA with 5% sheep blood to prepare isolates.

10.4 Preparation of V2C suspensions for Test Microbes and QC Organisms:

10.4.1 Using sterile cotton swabs, prepare a homogenous organism suspension by transferring several isolated colonies from the plates to 4 mL of sterile saline. Adjust the suspension to the McFarland standard required by the ID reagent using a calibrated V2C DensiChek Meter (e.g., 0.5-0.63 for GN and GP and 1.8-2.2 for BCL). See Reference 15.3. Place the prepared suspensions in the cassette.

NOTE: Suspensions not within the appropriate range on the VITEK 2 DensiChek may compromise the card performance. Re-calibrate the DensiChek instrument after processing each cassette (10 cards).

10.5 Selection and Inoculation of the V2C Card:

10.5.1 Select the appropriate card based on the Gram stain reaction and the organism's microscopic appearance.

10.5.2 Allow the card(s) to come to room temperature before opening the

package liner.

10.5.3 With the inoculated suspension tubes in the cassette, insert the straw from the appropriate V2C card into the suspension tube. Proceed to data entry.

NOTE: The age of the suspension must not exceed 30 minutes before inoculating the cards.

10.6 Data Entry into the VITEK system:

10.6.1 V2C is icon driven. When the system is initialized, an icon screen will appear with two rows of icons. To enter the test microbe/QC organism information in the application screen, double click the first icon on the left side, middle screen (the Manage Cassette View icon, which looks like the cassettes used to load the cards).

10.6.2 Click on Enter Manage Cassette View from the Main Menu.

10.6.3 Click on Maintain Virtual Cassette icon in the left view bar of the Setup Test Post Entry Window.

10.6.4 Click on Create New Virtual Cassette icon in the upper right view bar also called the Action Bar. The Maintain Virtual Cassette window appears. The Virtual Cassette stores the data scanned into the computer.

10.6.5 Enter the cassette information. You may either scan the cassette barcode or choose the number from the drop down window labeled cassette.

10.6.6 Enter the card data by scanning the bar code on the card. The Cursor must be in the Bar Code space to be entered. You may either hit ENTER and the cursor will move to the next line to be scanned or use the mouse button to move the cursor to the next Bar Code space.

10.7 Define Isolate Group Information in the Accession space:

10.7.1 For QC organisms the Accession number is the ATCC number and is automatically entered when you select the organism being QC'd.

10.7.2 For the product test organisms the Accession number will be the test coordinator's initials, the test date followed by an alpha-numeric

sequence and the tube number. The alpha numeric sequence will give the abbreviation of the test organism (Sa for *Staphylococcus aureus*, Pa for *Pseudomonas aeruginosa*, Bs for *Bacillus subtilis*, Uk for an unknown). For example if your test date is 01/21/06, the test coordinator is Jane Doe, the test organism is Pa and the tube number is 43/2, the accession number will be: JD012106Pa43/2-1. With the accession number, the computer automatically places a -1 at the end of the every accession number. This number cannot be removed.

NOTE: Checking the QC box will mark the card as a QC organism and the data for this card will be stored in a separate database.

10.7.3 Save the information. The save icon is in the upper right hand corner. Make sure you save your data prior to logging out or continuing to prepare additional isolates. Any unsaved data will not be recovered when the inactivity time limit (60 minutes) has been exceeded.

10.7.4 If you are logged on the system and you exceed the inactivity time limit, the application will automatically ask you to log in again when you attempt to use the application. If the inactivity time limit logs you out of the computer while you are entering data, you must reenter your data after you log back into the system.

10.8 Filling the Cards:

10.8.1 Place the cassette in the Filler box on the left side of the V2C unit and hit Start Fill button on the instrument. Filling the cards takes approximately 70 seconds for a cassette regardless of the number of cards in the cassette holder. The V2C instrument will beep when the filling cycle is complete.

[Discard individual cards that may have been exposed to multiple fill cycles as the test results will be inaccurate.]

NOTE: The V2C must be loaded within 10 minutes from the end of filling the cards to the start of loading the cards to avoid the cards from being rejected.

10.8.2 When the cards are finished filling, the Load Door is automatically unlocked. Place the cassette in the Load Door. The V2C Instrument will verify the scanned barcodes against the Virtual Cassette (the information scanned in by the analyst). Cards are sealed, straws are cut and the cards are loaded automatically into the carousel. The V2C will

beep once all cards are loaded into the cassette.

10.8.3 When the cards are loaded, remove the cassette and dispose of the tubes and straws in a biohazard container.

10.8.4 The V2C automatically proceeds to processing the cards once all the cards are loaded.

NOTE: Review the Navigation Tree. If the cassette status description in the Navigation Tree is red, the cassette needs more information to completely process the tests cards. Open up the red colored file and make sure all fields are defined. Red text may be an indication of an accession number not defined, a missing card, an extra card, or a wrong card.

10.8.5 When the cards are finished and results obtained, cards will be automatically ejected into the waste bin.

10.9 Results are concurrently printed and the data sent to the Results View folder on the left side of the screen also called the Navigation Tree where the information is archived. If an error occurs during processing, refer to section 4.5.1 or the On-line Reference Manual (See reference 15.1).

10.10 Review results printout and file with the appropriate notebooks. See section 12.1.

11.0 DATA ANALYSIS/CALCULATIONS:

11.1 The VITEK system analyses the data results and determines the identity of the test microbes/QC organism based on colorimetric tests (biochemical reactions).

11.2 Certain species may belong to a mixed (viewed as slashline) taxa identification. This occurs when the biopattern is the same for the taxa listed. Supplemental tests may be used to separate slashline taxa. Refer to the User Manual for information on slashline taxa differentiation for supplemental reaction recommendations.

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Data is automatically recorded and generated by the computer in the form of a printout. The printout for QC organisms will be filed in the VITEK Quality Control Record Book and the printout for any other test organism will be filed in the archive room with the raw data sheets. Information on Quality Control will be maintained in the VITEK Quality Control Record Book. Completed

forms and reports are archived in notebooks kept in secure file cabinets in file room D217. Only authorized personnel have access to the locked files. Archived data are subject to OPP's official retention schedule contained on SOP ADM-03, Records and Archives.

13.0 QUALITY CONTROL:

13.1 Quality Control (QC) procedures for the V2C encompass the annual service conducted by bioMérieux and the annual QC of one lot of each type of cards (GN, GP and BCL). The QC of the organism, VITEK card, and V2C are interdependent. Thus, each one individually or collectively can impact the identification of the organism.

13.1.1 A performance check of the VITEK unit will be performed on an annual basis by a trained bioMérieux technician. All service and recertification documents will be maintained in the VITEK Maintenance and Certification Records Book.

13.1.2 Internal Quality Control (QC) of the VITEK cards using all of the QC organisms listed in Table 1 will be conducted on an annual basis approximately at the half way point from the annual recertification date.

13.1.3 After the first transfer onto the appropriate media for the QC organisms, gram stain and colony morphology will be recorded on the VITEK 2 Compact: Test Microbe Transfer and Confirmation Sheet for Quality Control Organisms form.

13.1.3 The frozen QC organisms may be used until growth or VITEK performance shows a decline in viability.

Table 1. Quality Control Organisms for VITEK 2 COMPACT Automated Identification System

Organism	ATCC Number	Card Type	Media and Incubation Conditions	Identification Number
Streptococcus equi spp. zooepdiemicus	43079	GP	TSA or TSA with 5% sheep blood/35-37°C	V2C-02
Enterococcus casseliflavus	700327	GP	TSA or TSA with 5% sheep blood/35-37°C	V2C-03
Kocuria kristinae	BAA-752	GP	TSA or TSA with 5% sheep blood/35-37°C	V2C-04
Listeria monocytogenes	BAA-751	GP	TSA or TSA with 5% sheep blood/35-37°C	V2C-05
Staphylococcus aureus	29213	GP	TSA or TSA with 5% sheep blood/35-37°C	V2C-06
Staphylococcus saprophyticus	BAA-750	GP	TSA or TSA with 5% sheep blood/35-37°C	V2C-07
Staphylococcus sciuri	29061	GP	TSA or TSA with 5% sheep blood/35-37°C	V2C-08
*Streptococcus thermophilus**	19258	GP	TSA or TSA with 5% sheep blood/54-56°C	V2C-09
Proteus vulgaris	6380	GN	TSA or TSA with 5% sheep blood/35-37°C	V2C-10
Shigella sonnei	25931	GN	TSA or TSA with 5% sheep blood/ 35-37°C	V2C-11
Stenotrophomonas maltophilia	17666	GN	TSA or TSA with 5% sheep blood/35-37°C	V2C-12
Klebsiella oxytoca	700324	GN	TSA or TSA with 5% sheep blood/35-37°C	V2C-13
Acinetobacter baumannii	BAA-747	GN	TSA or TSA with 5% sheep blood/35-37°C	V2C-14
Enterobacter cloacae	700323	GN	TSA or TSA with 5% sheep blood/35-37°C	V2C-15
Ochrobactrum anthropi	BAA-749	GN	TSA or TSA with 5% sheep blood/35-37°C	V2C-16
Aneurinibacillus aneurinilyticus	11376	BCL	TSA or TSA with 5% sheep blood/35-37°C	V2C-17
Bacillus circulans	61	BCL	TSA or NA/30-37°C	V2C-18
Bacillus licheniformis	12759	BCL	TSA or NA/30-37°C	V2C-19
Brevibacillus agri	51663	BCL	TSA or NA/30-37°C	V2C-20
Brevibacillus laterosporus	64	BCL	TSA or NA/30-37°C	V2C-21
*Geobacillus stearothermophilus**	12978	BCL	TSA or NA/54-56°C	V2C-22
Paenibacillus macerans	8509	BCL	TSA or NA/30-37°C	V2C-23
Paenibacillus polymyxa	7070	BCL	TSA or NA/30-37°C	V2C-24
Virgibacillus pantothenticus	14576	BCL	TSA or NA/30-37°C	V2C-25

*Thermophiles

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 Refer to the On-Line and Instrument User Manual for response to Error Messages and any necessary corrective actions. See Section 4.5.for Error Message Queue.

15.0 REFERENCES:

15.1 bioMérieux VITEK, Inc. 2004. Vitek 2 Compact Online Software User Manual. Part Number: 510773-1EN1.

15.2 bioMérieux VITEK, Inc. 2004. Vitek 2 Compact Hardware User Manual. Part Number: 510772-1EN1.

15.3 bioMérieux VITEK, Inc. 2003. DensiChek User's Manual. Part Number: 93060 Version C.

16.0 FORMS AND DATA SHEETS:

16.1 VITEK 2 Compact: Microbe Transfer and Confirmation Sheet for Quality Control Organisms.

VITEK 2 COMPACT: MICROBE TRANSFER AND CONFIRMATION SHEET for QUALITY CONTROL ORGANISMS

OPP Microbiology Laboratory

QC Organism:		Identification Number**	V2C-
Source and Strain no.:	ATCC #	Notes:	

Date/Initials/ Time	Subculture Source	Media Information			Results			
		Name	Prep. No.	Inc. Time/ Temp.	Date/ Initials	Staining Results	Colony Characteristics	Comments

*Record Gram stain results: GPC = Gram Positive Cocci; GNR = Gram Negative Rods; GPR = Gram Positive Rods

**Identification Number from Table 1 in QC-22.